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Morphology and phylogeny of four new *Lactarius* species from Himalayan India

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Abstract —Four new species of *Lactarius* are described from Himalayan India. *Lactarius olivaceoglutinus, L. pyriodorus,* and *L. yumthangensis* belong to *L.* subg. *Piperites* and *L. indochrysorrheus* is closely related to some representatives of *L.* subg. *Russularia*. An ITS based phylogeny confirms the phylogenetic placement of the four new species, although the monophyly of neither *Lactarius* subg. *Piperites* nor *L.* subg. *Russularia* can be confirmed. The ITS data also suggest that the Indian species are closely related to some European and American species.

Key words — ectomycorrhizal fungi, macrofungi, *Russulaceae*, Sikkim

Introduction

After segregation of the well-known ectomycorrhizal milkcaps into *Lactarius* Pers. and *Lactifluus* (Pers.) Roussel (Buyck et al. 2008, 2010), *Lactarius* sensu novo comprises three subgenera (Verbeken & Nuytinck 2013): *L.* subg. *Piperites* (Fr. ex J. Kickx f.) Kauffman, *L.* subg. *Russularia* (Fr. ex Burl.) Kauffman, and *L.* subg. *Plinthogali* (Burl.) Hesler & A.H. Sm. *Lactarius* subg. *Piperites* can be characterized as follows: pileus sticky to slimy/glutinous (more rarely dry and shiny), often hairy and/or zonate or with watery spots; stipe dry or sticky, often scrobiculate (Heilmann-Clausen et al. 1998, Basso 1999). In contrast, representatives of *L.* subg. *Russularia* typically have dry (rarely somewhat sticky) caps and stipes and colours that are predominantly orange or various tinges of brown (Heilmann-Clausen et al. 1998).

Sikkim, a small (0.22% of the land surface) state in India, lies in Eastern Himalaya, which is part of the Himalaya Hotspot, one of the 34 Global Biodiversity Hotspots (www.biodiversityhotspots.org). Within a very small geographical area it is substantially diverse in flora, fauna, and mycobiota, the latter seriously underexplored. During mycological expeditions to different temperate to subalpine areas of the North District of Sikkim in 2009 (KD & AV), 2011 (KD), and 2012 (KD), we collected ectomycorrhizal (ECM) fungi, with *Lactarius* s.l. one of the dominant ECM genera in Dombang, Shingba Rhododendron Sanctuary, and Zema. Dombang is a subalpine coniferous to mixed (coniferous & broadleaf) forest dominated by *Picea spinulosa*, *Abies densa*, *Tsuga dumosa*, and *Larix griffithii*. Shingba Rhododendron Sanctuary contains subalpine coniferous and mixed forests distributed in Yumthang valley and its adjoining areas; apart from several *Rhododendron* species, this protected area is dominated by *Abies densa*, *Picea spinulosa*, *Tsuga dumosa, Larix griffithii*, *Magnolia globosa*, *M. campbellii*, *Acer pectinatum*, and *Betula utilis*. Zema is a subalpine coniferous forest dominated by *Abies densa*.

Although Sikkim harbours a large number of russulaceous taxa, only 32 species (out of ca 210 taxa recorded from India) have been reported (Berkeley 1852; Das 2009; Das et al. 2010, 2013; Das & Verbeken 2011, 2012; Van de Putte et al. 2012). We describe here three new species of *Lactarius* subg. *Piperites* (*L. olivaceoglutinus, L. pyriodorus*, and *L. yumthangensis*) and one new species of *L.* subg. *Russularia* (*L. indochrysorrheus*).

Materials & methods

Morphological study

Macromorphological characters were described from the fresh basidiomata in daylight. Colour codes and terms follow the Colour identification chart of British Fungus Flora (Henderson et al. 1969, here prefixed by "a:") or the Methuen Handbook of Colour (Kornerup & Wanscher 1981, here prefixed by "b:"). Spore print colour codes follow Kränzlin (2005, here prefixed by "c:").

Micromorphological structures such as basidia, hymenial cystidia, pileipellis, stipitipellis, etc. were observed from free-hand sections of dry samples mounted in a mixture of 5% KOH, 30% Glycerol, Phloxine, and Cotton Blue using an Olympus CX41 compound microscope. Spores and spore ornamentation were studied in Melzer's reagent. Drawings were made from SEM images obtained in different magnifications. Spore measurements were calculated based on 20 basidiospores per specimen; dimensions represent minimum–**mean**–maximum length × minimum–**mean**–maximum width, and Q = length/width ratio. Herbarium names are after Holmgren et al. (1990).

DNA extraction, PCR amplification and sequencing

DNA was extracted from dried fruiting bodies according to Nuytinck & Verbeken (2003) with slight modifications (Van de Putte et al. 2010). The internal transcribed

Table 1. Collections of *Lactarius* and allied species used for molecular analyses.

Species	Voucher	Herbarium	Origin	Genbank
Lactarius akahatsu	Verbeken 04-141	GENT	Thailand	KF133269
L. albocarneus	Verbeken 98-071	GENT	France	KJ742389
	Verbeken 98-080	GENT	France	KF241545
L. alboscrobiculatus	Le 175	CMU, SFSU, GENT	Thailand	EF141538
L. aspideus	Walleyn 3815	GENT	Czech Rep.	KJ742390
L. atroviridis	Verbeken 05-306	GENT	USA	KF133270
L. auriolla	Walleyn 1601	GENT	Sweden	KF133257
L. indochrysorrheus	KD 11-002 (Holotypus)	BSHC, GENT	India	KJ742391
L. azonites	Verbeken 00-124	GENT	Belgium	KF241540
L. brunneoviolaceus	Verbeken 04-220	GENT	France	KJ742392
	Verbeken 04-249	GENT	France	KJ742393
	Walleyn 1605	GENT	Sweden	KJ742394
	Eberhardt 24.08. 04-8	UPS	Sweden	KJ742395
L. caespitosus				FJ845421
L. camphoratus	Oberwinkler 46773	TUB	Germany	AY606945
L. chichuensis	Wang 1236	HKAS	China	KF475766
L. chrysorrheus	Nuytinck 01-089	GENT	Belgium	KJ742396
	D'Hooge 08-020	GENT	France	KJ742397
	Eberhardt 04.10. 02-8	UPS	Italy	KF133261
L. aff. *chrysorrheus*	Verbeken 04-212	GENT	USA	KJ742398
	Verbeken 05-359	GENT	USA	KJ742399
L. citriolens	Eberhardt 20.09.04-3	UPS	Sweden	DQ422003
L. controversus	Verbeken 00-117	GENT	Italy	KF241544
L. crassiusculus	Le 369	CMU, SFSU	Thailand	EF560684
L. cyathuliformis	Eberhardt 04.09.04-3	UPS	Sweden	KF133266
L. flexuosus	Walleyn 2136	GENT	Sweden	KJ742400
	Walleyn 3178	GENT	Czech Rep.	KJ742401
	Eberhardt 06.09.02-1	UPS	Sweden	DQ421992
L. formosus	Le 382 (Holotypus)	CMU, SFSU, GENT	Thailand	EF141549
L. fuliginosus	Basso 97-24	priv. herb.	Sweden	JQ446111
L. helvus	Eberhardt 08.09. 04-1	UPS	Sweden	KF133263
L. lilacinus	Walleyn 3774	GENT	Belgium	KF133275
L. luridus	Taylor 2003066	UPS	Sweden	KJ742402
	Eberhardt 17.09.04-3	UPS	Sweden	KJ742403
	Eberhardt 10.10. 04-4	UPS	Sweden	KJ742404
	Berteloot 11-011	GENT	Belgium	KF241547
	Berteloot 11-012	GENT	Belgium	KJ742405
	Taylor 2004254	UPS	Sweden	KJ742406
	Walleyn 1455	GENT	Belgium	KJ742407
L. montoyae	KD 1065 (Holotypus)	BSD	India	EF560673
L. necator	Verbeken 04-231	GENT	France	KF133276
L. olivaceoglutinus	KD 11-103 (Holotypus)	BSHC, GENT	India	KJ742408
L. pallescens				DQ974747
L. peckii	Nuytinck 04-020	GENT	USA	KF133277
L. pseudouvidus	Eberhardt 24.08. 04-14	UPS	Sweden	KJ742409
	Eberhardt 24.08. 04-13	UPS	Sweden	KJ742410

L. pubescens	Eberhardt 15.09.02-2	UPS	Sweden	DQ421996
L. pyriodorus	KD 11-027 (Holotypus)	BSHC, GENT	India	KJ742411
L. quieticolor	Eberhardt 10.09.04-1	UPS	Sweden	DQ422002
L. quietus	Eberhardt 16.09.04-6	UPS	Sweden	KF133264
L. rufus	Nuytinck 02-008	GENT	Norway	KF241543
L. sphagneti	Walter 083	TUB	Germany	KJ742412
L. subdulcis	Vervisch 06-024	GENT	Belgium	KF133279
L. subplinthogalus	Verbeken 04-219	GENT	USA	KF241539
L. subsericatus	Eberhardt 11.10.04-8	UPS	Sweden	DQ422011
L. thyinos	Voitk 23-08-04	priv. herb.	Canada	KF133271
L. torminosus	Walleyn 3183	GENT	Czech Rep.	KF133281
L. trivialis	Van de Putte 10-011	GENT	Russia	KJ742413
	Walleyn 3179	GENT	Czech Rep.	KJ742414
	Eberhardt27.08. 02-17a	UPS	Sweden	DQ421991
L. uvidus	Van de Putte 10-027	GENT	Russia	KF241546
	Nuytinck 01-033	GENT	Finland	KJ742415
	Walleyn 1237	GENT	France	KJ742388
	Walleyn 2119	GENT	Sweden	KJ742416
	Eberhardt 28.08. 02-24	UPS	Sweden	KJ742417
L. vietus	Eberhardt 11.10.04-1	UPS	Sweden	KF133267
L. vinaceorufescens	Nuytinck 07-018	GENT	Canada	KF241542
L. yumthangensis	KD 11-147 (Holotypus)	BSHC, GENT	India	KJ742418
Lactifluus volemus	Eberhardt 09.08. 04-5	UPS	Sweden	DQ422008
Lactifluus vellereus	Eberhardt 20.09.04-22	UPS	Sweden	DQ422034
Multifurca ochricompacta	Buyck 02-107	PC	USA	DQ421984
M. zonaria	Desjardin 7442	SFSU, PC, BBH	Thailand	DQ421990
Russula cyanoxantha	Eberhardt 29.09. 02-2	UPS	France	DQ422033
R. nigricans	Eberhardt 20.09.04-7	UPS	Sweden	DQ422010

spacer region of the nuclear ribosomal DNA (ITS) was amplified and sequenced using primers ITS1-F and ITS4 (White et al. 1990, Gardes & Bruns 1993). PCR amplification protocols follow Le et al. (2007); sequencing was conducted with an ABI 3730XL or ABI 3700 by Macrogen (Amsterdam, The Netherlands). Sequences were assembled and edited with Sequencher™ v4.9 (GeneCodes Corporation, Ann Arbor, Michigan, U.S.A.). Specimens and sequences used in the phylogenetic analysis are shown in Table 1.

Alignment and phylogenetic analyses

Alignment was conducted with the on-line version of MAFFTv7 (Katoh & Standley 2013), applying the E-INS-I strategy, a very slow method recommended for fewer than 200 sequences with multiple conserved domains and long gaps. The alignment was manually refined in BioEdit v7.0.9.0 (Hall 1999). Ambiguously aligned positions were detected using Gblocks v0.91b (Castresana 2000), specifying less stringent conditions than default. The minimum number of sequences for a conserved position and for a flank position was set to half the number of sequences, the number of contiguous non-conserved positions was set to 10, and the minimum length of a block after gap cleaning to 5 and positions with gaps were not treated differently from other positions. The ITS

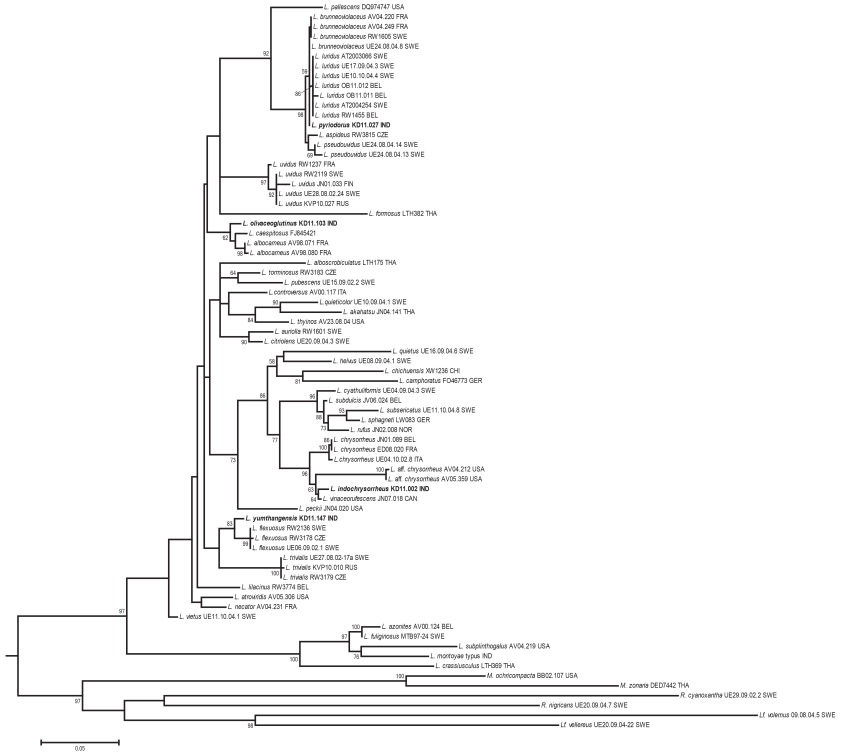
L. pallescens DQ974747 USA
L. brunneoviolaceus AV04.220 FRA
L. brunneoviolaceus AV04.249 FRA
L. brunneoviolaceus RW1606 SWE
L. brunneoviolaceus UE24.09.04.8 SWE
L. luridus AT2003066 SWE
L. luridus UE17.09.04.3 SWE
L. luridus UE10.10.04.4 SWE
L. luridus OB11.012 BEL
L. luridus OB11.011 BEL
L. luridus AT2004254 SWE
L. luridus RW1455 BEL
L. pyriodorus KD11.027 IND
L. aspideus RW3815 CZE
L. pseudouvidus UE24.09.04.14 SWE
L. pseudouvidus UE24.09.04.13 SWE
L. uvidus RW1237 FRA
L. uvidus RW2119 SWE
L. uvidus JN01.033 FIN
L. uvidus UE28.08.02.24 SWE
L. uvidus KVP10.027 RUS
L. flavidus LTH382 THA
L. olivaceoglutinus KD11.103 IND
L. caespitosus FJ845421
L. albocarneus AV98.071 FRA
L. albocarneus AV98.080 FRA
L. albocrocicolatus LTH175 THA
L. torminosus RW3183 CZE
L. pubescens UE15.09.02.2 SWE
L. controversus AV00.117 ITA
L. quietior UE10.09.04.1 SWE
L. akahatsu JN04.141 THA
L. thyiosa AV23.08.04 USA
L. aurora RW1601 SWE
L. citriolens UE20.09.04.3 SWE
L. quietus UE16.09.04.6 SWE
L. helus UE08.09.04.1 SWE
L. chrysorheus RW1236 CHI
L. camphoratus FO48773 GER
L. cyathuliformis UE04.09.04.3 SWE
L. subdulcis JV06.024 BEL
L. subserialis UE11.10.04.8 SWE
L. sphagneti LW083 GER
L. rufus JN02.008 NOR
L. chrysorheus JN01.089 BEL
L. chrysorheus ED06.020 FRA
L. chrysorheus UE04.10.02.8 ITA
L. aff. chrysorheus AV04.212 USA
aff. chrysorheus AV05.359 USA
L. indochrysorheus KD11.002 IND
L. vinaceorufescens JN07.018 CAN
L. peckii JN04.020 USA
L. yumthangensis KD11.147 IND
L. flexuosus RW2136 SWE
L. flexuosus RW3178 CZE
L. flexuosus UE09.09.02.1 SWE
L. mitis UE27.08.02-17a SWE
L. mitis KVP10.010 RUS
L. mitis RW3179 CZE
L. lacunus RW3774 BEL
L. atrowirdis AV05.306 USA
L. necator AV04.231 FRA
L. vietus UE11.10.04.1 SWE
L. azonites AV00.124 BEL
L. fulgineus MTB97-24 SWE
L. subplinthogalus AV04.219 USA
L. montoyae typus IND
L. crassusculus LTH969 THA
M. ochricompacta BB02.107 USA
M. zonaria DED7442 THA
R. cyanoxantha UE29.09.02.2 SWE
R. nigricans UE20.09.04.7 SWE
Lf. volemus 09.08.04.5 SWE
Lf. vellereus UE20.09.04-22 SWE
0.05

Plate 1. The obtained ML topology based on ITS sequences of *Lactarius, Multifurca, Russula,* and *Lactifluus* species. Bootstrap values >50% are indicated. Names in bold are the new Indian species described in this paper. The scale bar represents the number of nucleotide changes per site.

sequences were partitioned into 5 partitions: the ribosomal genes 18S, 5.8S, and LSU and the spacer regions ITS1 and ITS2.

Maximum Likelihood (ML) analysis was performed in RAxML v7.0.3 (Stamatakis 2006), combining a ML search with the Rapid Bootstrapping algorithm for 1000 replicates. The model GTRGAMMA was estimated for each partition separately.

Results

Phylogeny

Plate 1 shows the obtained ML topology with bootstrap support (BS) values >50% displayed. The tree shows a well-supported genus *Lactarius* (97% BS). Within *Lactarius, L.* subg. *Plinthogali* receives a high support value (100% BS), but monophyly of neither *Lactarius* subg. *Piperites* nor *L.* subg. *Russularia* (sensu Heilmann-Clausen et al. 1998) is supported. *Lactarius pyriodorus, L. olivaceoglutinus,* and *L. yumthangensis* are closely related to species

traditionally placed in *L.* subg. *Piperites*. *Lactarius indochrysorrheus* is closely related to the European *L. chrysorrheus* Fr. and the North American *L. vinaceorufescens* A.H. Sm. and *L.* aff. *chrysorrheus*, which are assigned to *L.* subg. *Russularia* in morphology-based taxonomy.

Species delimitation for this paper is mainly based on morphological features. We were not able to include enough specimens/sequences from the newly proposed species to test species delimitation in the phylogenetic tree.

Taxonomy

Lactarius indochrysorrheus K. Das & Verbeken, **sp. nov.** Plates 2, 3, 6a,b,e

MycoBank MB804887

Differs from *Lactarius chrysorrheus* by its more viscid pileus with its pileipellis an ixocutis covered by a thick gelatinous layer.

Type: India. Sikkim: North District, Dombang, 27°43′35.2″N 88°45′15.2″E, alt. 2920 m, 18.VIII.2011, K. Das, KD 11-002 (**Holotype**, BSHC; **isotype**, GENT).

Etymology: an Indian look-alike of the European *Lactarius chrysorrheus*.

Pileus 25–70 mm diam., convex with slightly pubescent and inrolled margin when young, gradually planoconvex to applanate with slightly depressed centre, rarely with a central papilla, sometimes becoming widely infundibuliform; margin decurved with maturity, becoming very irregularly wavy; surface smooth to greasy, viscid (sticky), shiny, brownish orange, pinkish buff to salmon (a: 45) or orange (b: 6A6) (cinnamon (a: 10) to somewhat rusty after maturity), gradually apricot cream to paler up to yellow (a: 5E), towards margin distinctly zonate with several zones over the whole diameter but most dense in the center; zones consisting of darker and watery spots; margin very faintly and shortly striate. Lamellae subdecurrent, crowded (18–22/cm at pileus margin), pale yellow (a: 4D) when young, gradually pink-spotted, finally becoming brown to reddish brown, with lamellulae in 9 series; edge entire, concolorous. Stipe 45–65 × 6–8.5 mm, slender, subcylindrical to cylindrical or slightly widened towards base; surface smooth, slightly greasy, strigose (hairy) at base, much paler than the pileus, especially in the upper part (very pale pinkish), very pale salmon to vinaceous, gradually darker up to rust to rusty tawny or pale brick red. Context hollow in stipe, pinkish yellow (a: 4D) to pale salmon, turning lemon yellow (a: 54) to greenish yellow (a: 57) after cut, changing to salmon (a: 45) with $FeSO_4$, and greenish with Guaiac. Latex abundant, white, turning quickly to greenish yellow. Taste first mild, then becoming bitter and acrid. Odour not distinctive. Spore print pale cream (c: 10 Y).

Basidiospores 6.4–**7.4**–8.5 × 5.6–**6.2**–6.9 µm, (Q = 1.08–**1.18**–1.33), subglobose to ellipsoid; ornamentation amyloid, ≤0.9 µm high, composed of conical to spine-shaped warts, with short or medium, irregular to regular

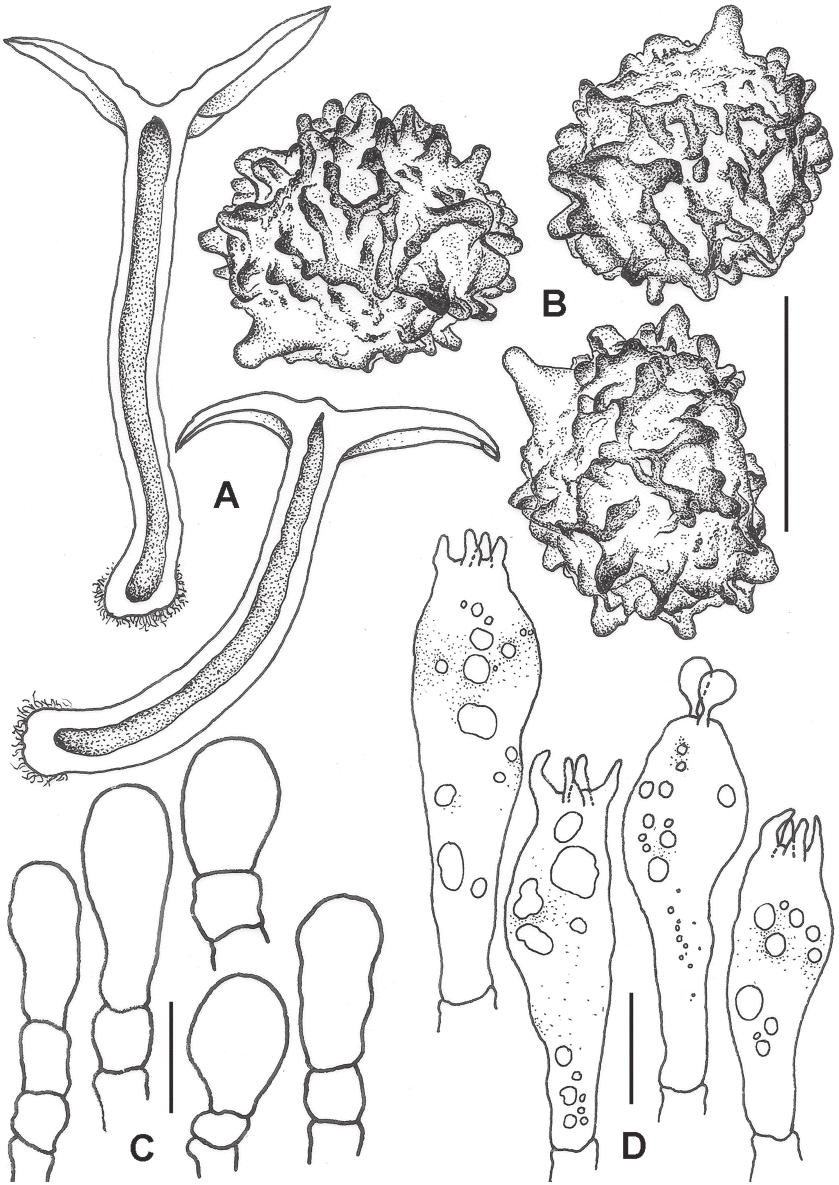

Plate 2: *Lactarius indochrysorrheus* (Holotype KD 11-002).
A. Fresh basidiomata showing lamellae and lamellulae. B. Basidiospores.
C. Marginal cells. D. Basidia. Scale bars: B = 5 µm; C, D = 10 µm.

ridges which are aligned or connected and forming a partial to incomplete reticulum; some isolated small warts present; plage sometimes distinct and amyloid. Basidia 30–44 × 9–13 µm, 4-spored, subclavate to ventricose; sterigmata 2.5–4 × 1.5–2 µm. Pleuromacrocystidia 32–70 × 7.5–11 µm, fairly abundant, emergent ≤10–30 µm, narrowly clavate, cylindric with tapered apex or fusoid, often with mucronate to subcapitate apex, slightly thick-walled (wall ≤0.5 µm); content refractive. Pleuropseudocystidia filamentous, 3–5 µm wide. Lamellar edge fertile with basidia, cystidia and marginal cells. Cheilomacrocystidia 35–55 × 8–9 µm, moderately abundant, subclavate to narrowly clavate or ventricose to fusoid sometimes with mucronate apex; content refractive. Cheilopseudocystidia filamentous. Marginal cells 11–20 × 7–11 µm, mostly clavate to subclavate, often multiseptate. Hymenophoral trama with lactifers. Pileipellis an ixocutis, ≤170 µm thick, with gelatinous layer mostly extended 10–15 µm beyond the hyphal layer; hyphae repent to suberect, ≤3.5 µm wide, branched, septate. Stipitipellis an ixocutis, composed of repent hyphae mostly in parallel pattern; hyphae ≤3.5 µm wide, branched, septate. Stipe trama mostly with numerous nested sphaerocytes. Clamp connections absent.

Ecology & Distribution — Gregarious under *Abies densa* and *Picea spinulosa* in subalpine coniferous or mixed (coniferous and broadleaf) forest. August. Fairly common.

Additional specimens examined: **India**. **Sikkim**: North District, Dombang, 27°43′35.2″N 88°45′15.2″E, alt. 2920 m, 14.VIII.2009, A. Verbeken, K. Das & K.V. Putte, AV-KD-KVP 032 (BSHC, GENT); North District, Shingba Rhododendron sanctuary, 27°43′42.1″N 88°44′58.8″E, alt. 2889 m, 24.VIII.2011, K. Das, KD 11-082 (BSHC); 27°44′03.5″N 88°44′23.3″E, alt. 3208 m, 26.VIII.2012, K. Das, KD 11-107 (BSHC).

Notes — Zonate species with similar cap colours and milk that stains quickly sulphur to greenish yellow are European *L. chrysorrheus*, North American *L. vinaceorufescens,* and an undescribed American species [labeled *L.* aff. *chrysorrheus* in the phylogenetic tree and Table 1]. ITS sequence comparison suggests the new species described here is close to the three species but not conspecific.

Lactarius chrysorrheus is less sticky, which is reflected in the pileipellis structure, a loosely interwoven cutis without distinct slime layer (Heilmann-Clausen et al. 1998). *Lactarius vinaceorufescens* differs by a pileipellis with a relatively thin ixocutis and the lack of ixocutis in the stipitipellis (Hesler & Smith 1979). *Lactarius xanthogalus* Verbeken & E. Horak, described from Papua New Guinea (Verbeken & Horak 2000), has a zonate orange to paler cap and latex that becomes immediately sulphur yellow but clearly differs by the zebroid ornamented spores.

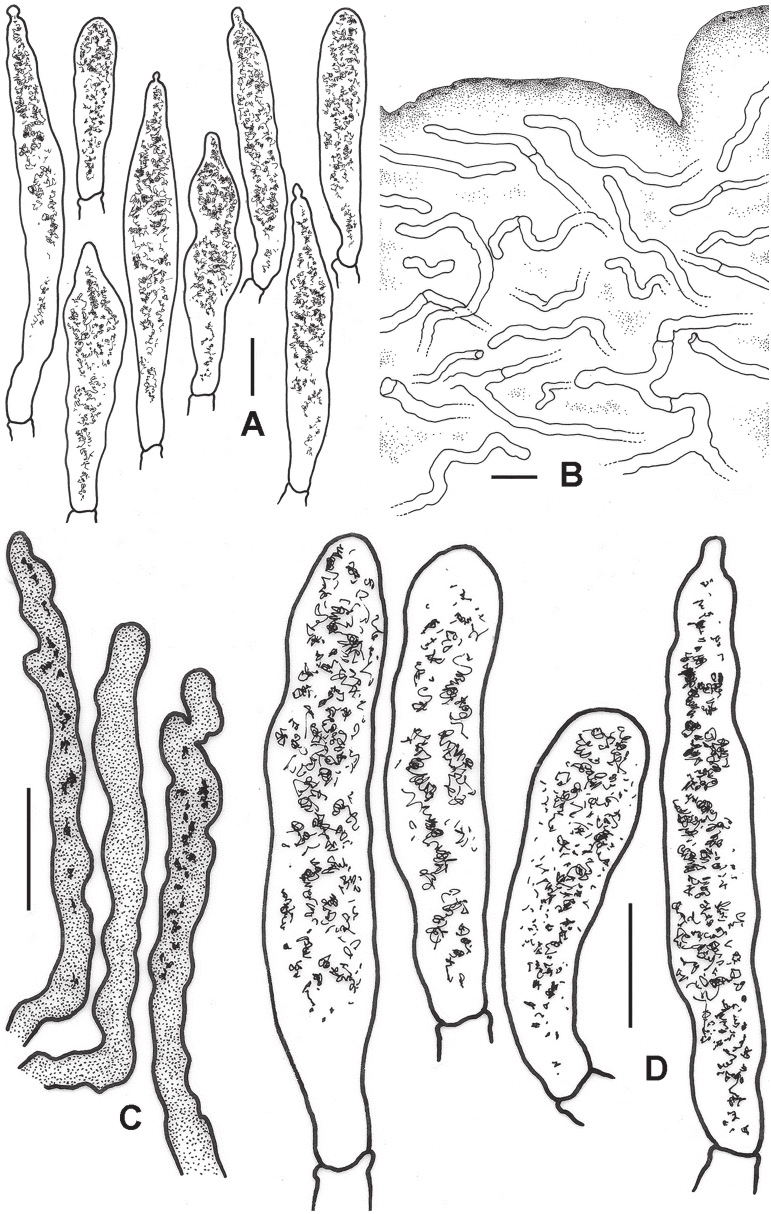

Plate 3. *Lactarius indochrysorrheus* (Holotype KD 11-002).
A. Pleuromacrocystidia. B. Radial section through pileipellis.
C. Pleuropseudocystidia. D. Cheilomacrocystidia. Scale bars = 10 µm.

Lactarius olivaceoglutinus K. Das & Verbeken, **sp. nov.** Plates 4, 5, 6c,d,f

MycoBank MB 804888

Differs from *Lactarius albocarneus* by its olivaceous pileus and its watery white latex that turns faintly pinkish on bruised or cut gill tissue.

Type: India. Sikkim: North District, Shingba Rhododendron sanctuary, 27°44′19.5″N 88°44′25.9″E, alt. 3252 m, 26.VIII.2011, K. Das, KD 11-103 (**Holotype**, BSHC; **isotype**, GENT).

Etymology: named after the olive and extremely glutinous pileus of the basidiocarps.

Pileus 40–68(–120) mm diam., at first convex, gradually becoming planoconvex with slightly depressed centre; surface smooth, viscid to highly glutinous, olive yellow (b: 3D8) to olive brown (b: 4E8, 4F6) or golden brown (b: 5D7) to greenish grey, grey-olive or darker towards centre and paler margin, azonate; margin non-striate, incurved when young, decurved with maturity. Lamellae adnexed to broadly adnate, crowded (17–18/cm at margin), with lamellulae in 5–6 series, pale cream to cream; edge entire, concolorous. Stipe 40–75 × 12–17 mm, clavate to subclavate (broader towards base) or ventricose (narrower towards apex and base); surface smooth or sometimes with shallow depressions (but without scrobicules), sticky, pale salmon to pale yellow (b: 3A3 to 4A3), gradually whitish towards base; base strigose, pale cream to white, sometimes with ochraceous spots. Context firm, stuffed to hollow in stipe, cream (a: 4D) to pale cream, unchanging when exposed or with KOH, changing to olivaceous (a: 62) to leaf green (a: 59) with Guaiac and pale green with $FeSO_4$. Latex whitish, very watery, turning faintly pinkish on bruised or cut lamellae. Taste mild and agreeable or slightly acrid. Odour indistinct. Spore print pale yellow (c: 30Y, 2M).

Basidiospores 8.0–**9.3**–10.1(–11) × 6.7–**7.3**–7.8(–8.4) µm, [Q = 1.16–**1.28**–1.33(–1.44)], broadly ellipsoid to ellipsoid; ornamentation amyloid, ≤1.2 µm high, composed of narrow, low and high ridges aligned and parallel, forming zebroid pattern, some connected with adjacent ones (but never forming a true reticulum), with each high ridge bearing elongated to conical (with rounded apex) warts; plage indistinct. Basidia 40–60 × 11.5–13 µm, 2–4-spored, ventricose; sterigmata 6–9 × 2–2.5 µm. Pleuromacrocystidia 57–88 × 8–12 µm, abundant, subcylindric or subclavate to fusiform with rounded, mucronate to moniliform (rarely) apex, emergent ≤41 µm; content dense. Pleuropseudocystidia cylindrical to tortuous, rare, never emergent, 4–5 µm wide; content refringent. Lamellar edge sterile with marginal cells and some cystidia. Cheilomacrocystidia 32–57 × 7–9 µm, subcylindrical to subfusiform, mostly with rounded apex. Marginal cells 13–23 × 6.5–9 µm, narrowly clavate to clavate or subcapitate, slightly thick-walled, hyaline. Hymenophoral trama with lactifers. Pileipellis an ixotrichoderm, very thick (often variable in thickness), 220–700 µm thick, composed of erect,

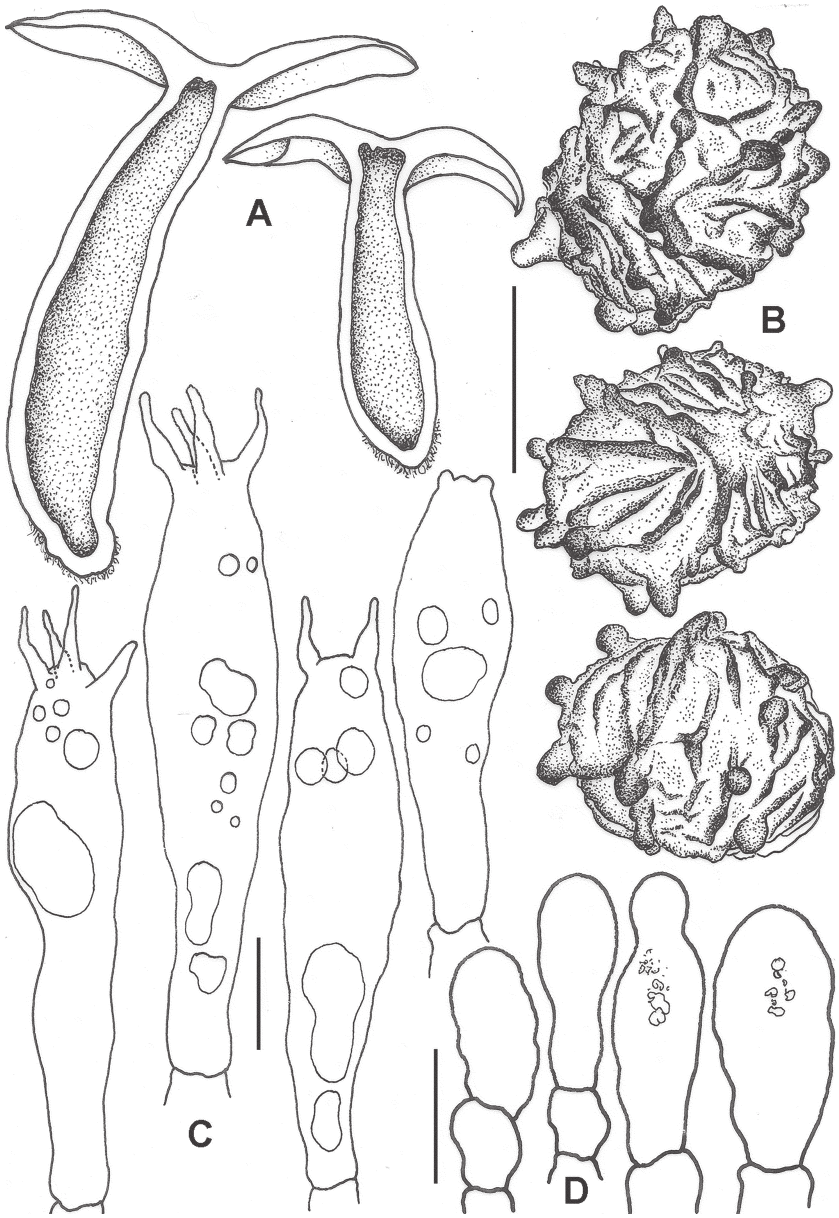

Plate 4. *Lactarius olivaceoglutinus* (Holotype KD 11-103).
A. Fresh basidiomata showing lamellae and lamellulae. B. Basidiospores.
C. Basidia. D. Marginal cells. Scale bars: B = 5 μm; C, D = 10 μm.

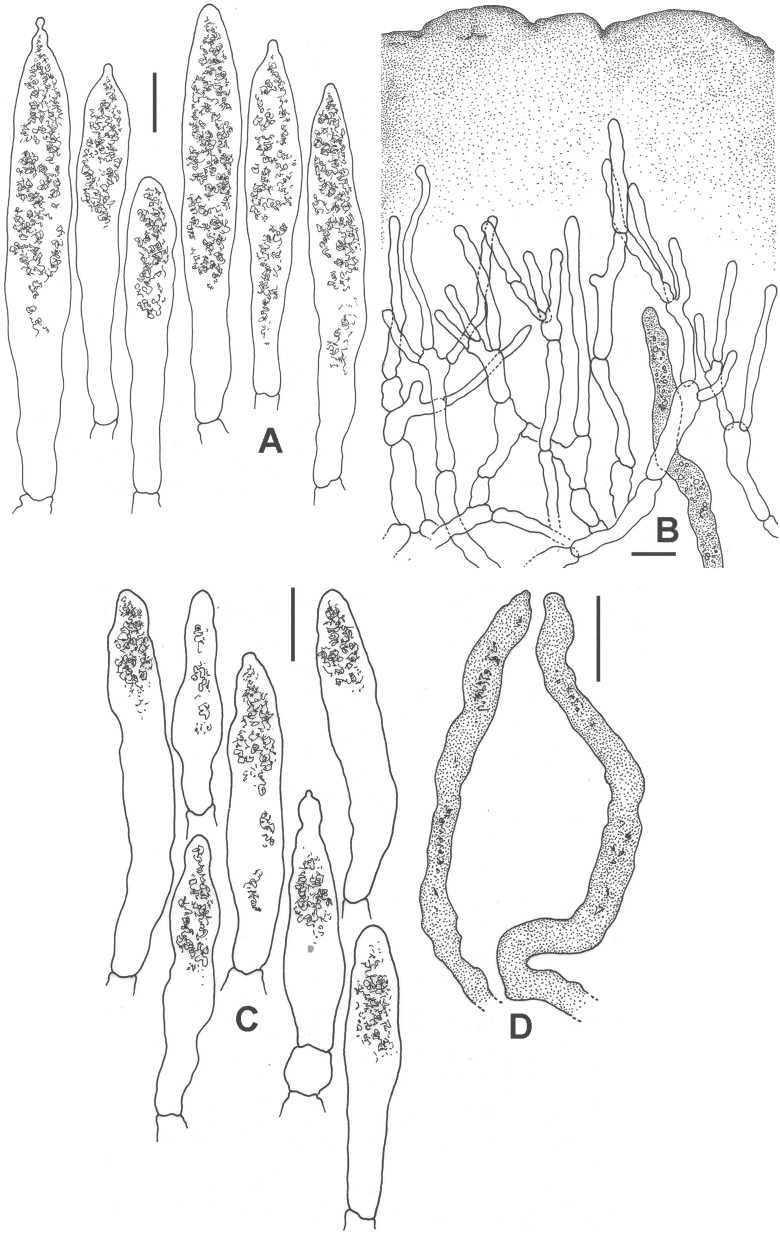

Plate 5. *Lactarius olivaceoglutinus* (Holotype KD 11-103).
A. Pleuromacrocystidia. B. Radial section through pileipellis.
C. Cheilomacrocystidia. D. Pleuropseudocystidia. Scale bars = 10 µm.

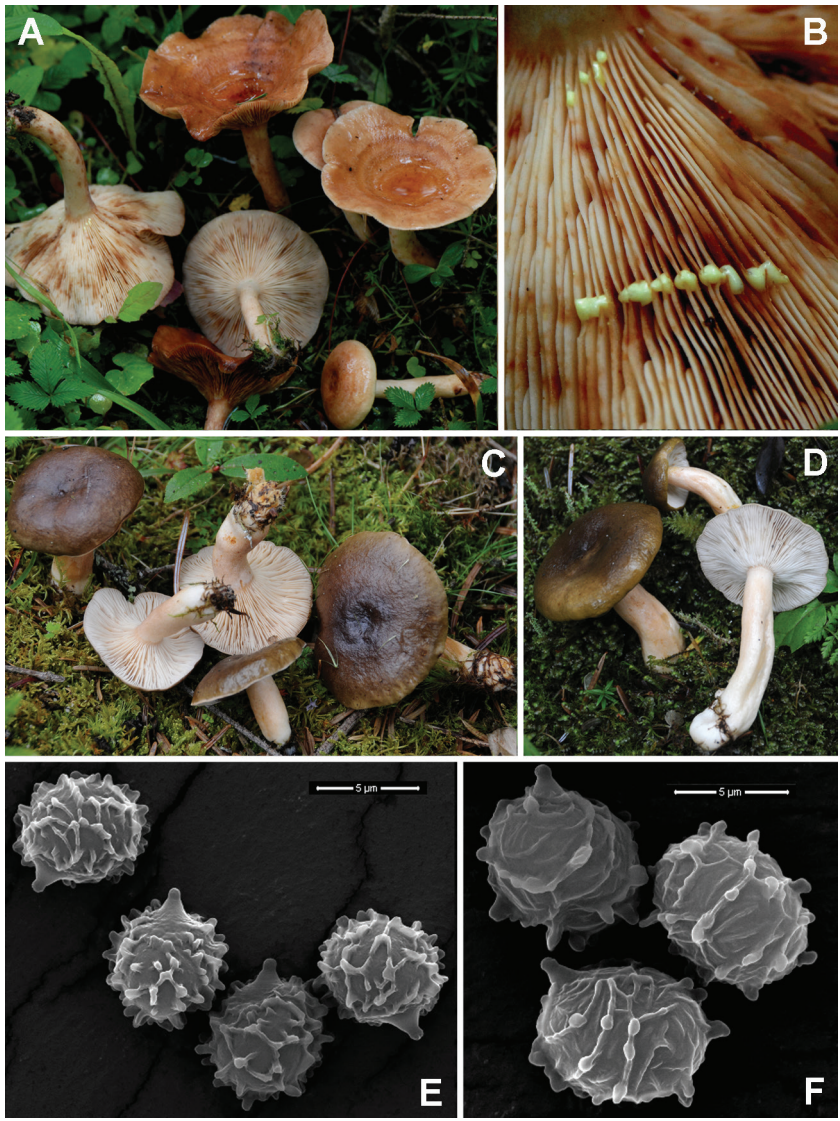

Plate 6. ***Lactarius indochrysorrheus***. A. Fresh basidiomata. B. Latex oozing from cut lamellae. E. Basidiospores (SEM). ***Lactarius olivaceoglutinus***. C, D. Fresh basidiomata. F. Basidiospores (SEM). Scale bars: E, F = 5 µm.

branched multi-septate hyphae (≤3.5 µm wide) and few lactifers embedded in a slime layer of variable thickness underlying somewhat repent hyphae. Stipitipellis an ixotrichoderm, ≤120 µm thick, composed of erect branched

septate hyphae (≤3 µm wide) embedded in a slime layer. Stipe trama with numerous nested sphaerocytes. Clamp connections absent.

Ecology & Distribution — Gregarious under *Abies densa* in subalpine coniferous or mixed (coniferous and broadleaf) forest. August–September. Fairly common.

Additional specimens examined: **INDIA**. **Sikkim**: North District, Shingba Rhododendron sanctuary, 27°45′11.1″N 88°43′47.5″E, alt. 3580 m, 29.VIII.2011, K. Das, KD 11-144 (BSHC); North District, Dombang, 27°44′53.1″N 88°44′58.8″E, alt. 3058 m, 03.IX.2011, K. Das, KD 11-191 (BSHC); 27°44′51″N 88°46′32″E, alt. 2940 m, 15.VIII.2009, A. Verbeken, K. Das & K.V. Putte, AV-KD-KVP 09-049 (BSHC, GENT).

Notes — The combination of the viscid to glutinous pileus and stipe and the ixotrichoderm pileipellis clearly places the present species in *L.* sect. *Pallidini*. *Lactarius olivaceoglutinus* can be recognized by its distinctly glutinous grey-olive to greenish grey pileus, pale salmon to pale yellow sticky stipe, whitish watery latex that turns the bruised lamellae faintly pinkish, large spores with mostly zebroid patterns of ridges (that carry warts), and its occurrence under *Abies* sp. ITS sequences and morphology (mainly the thick pileipellis ixotrichoderm) suggest a close relationship with the European *L. albocarneus* Britzelm. [= *L. glutinopallens* F.H. Møller & J.E. Lange] and North American *L. caespitosus* Hesler & A.H. Sm. The pileus of *L. albocarneus* however, is dominantly cream to at most olivaceous buff and its latex slowly turns to sulfur-yellow (Heilmann-Clausen et al. 1998, Kränzlin 2005) and its spore ridges never bear elongated to conical warts. *Lactarius caespitosus* also has a viscid cap with similar grey to olive brown colours, but its spores are slightly larger with a distinctly low and non-zebroid ornamentation (Hesler & Smith 1979).

In Sikkim *L. elaioviscidus* K. Das & Verbeken is another species with a glutinous olivaceous pileus and watery white latex (Das & Verbeken 2011); however, it is distinguished from *L. olivaceoglutinus* by the presence of a papilla, its greyish yellow to yellowish white stipe without pinkish tinges, latex changing to yellow-yellowish green after long exposure, and smaller basidiospores without zebroid ornamentation (7.0–**8.1**–8.7 × 6.0–**6.7**–7.1 µm).

Lactarius pyriodorus K. Das & Verbeken, **sp. nov.** Plate 7–9, 13a,b,f

MycoBank MB 804889

Differs from *Lactarius formosus* by lacking bundles of glutinous hairs on the pileus.

Type: India. Sikkim: North District, Dombang, 27°44′08.7″N, 88°45′58.3″, alt. 2975 m, 19.VIII.2011, K. Das, KD 11-027 (**Holotype**, BSHC; **isotype**, GENT).

Etymology: in reference to the distinct pear-like odor of the fresh basidiocarps.

Pileus 35–77 mm diam., at first convex with slightly depressed centre, gradually becoming planoconvex with depressed centre and finally broadly to widely infundibuliform, rarely with a small, sometimes blunt central papilla; surface

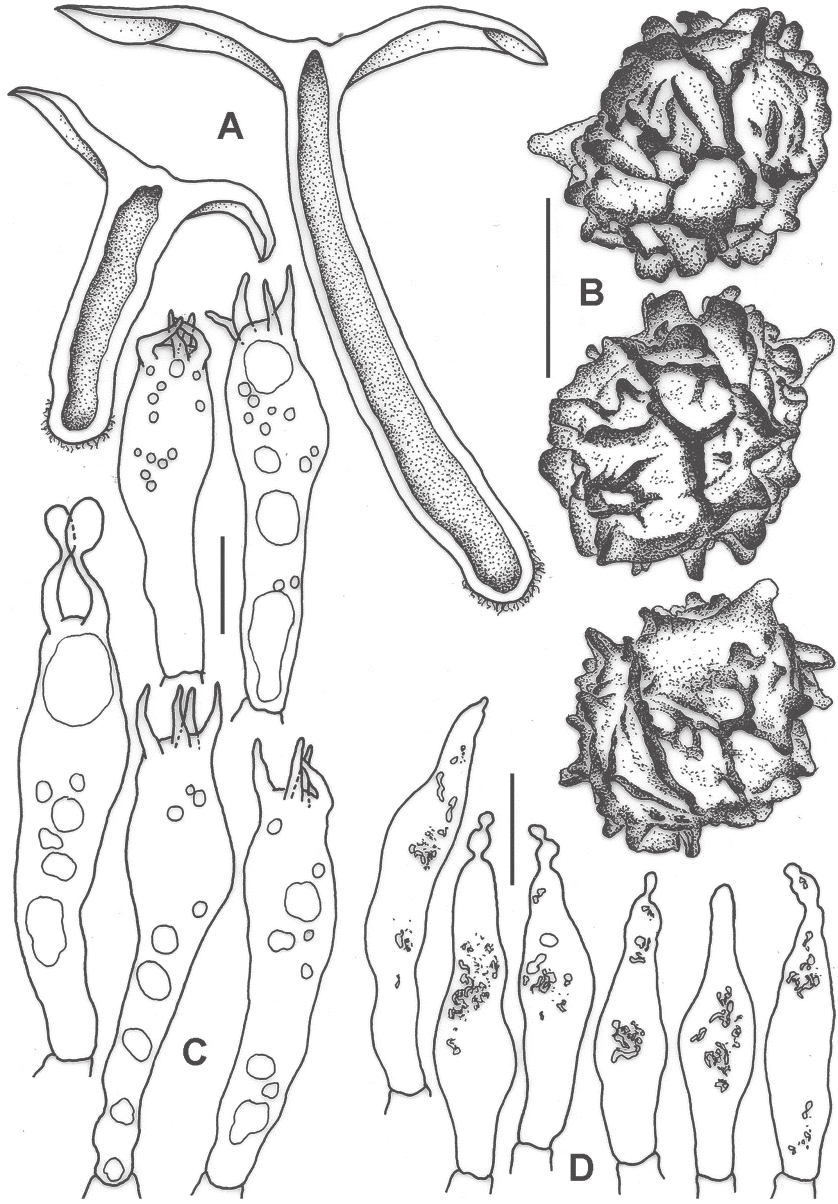

Plate 7. *Lactarius pyriodorus* (Holotype KD 11-027).
A. Fresh basidiomata showing lamellae and lamellulae. B. Basidiospores.
C. Basidia. D. Cheilomacrocystidia. Scale bars: B = 5 μm; C, D = 10 μm.

smooth to finely rugulose under the slimy surface, viscid to highly glutinous and slimy, mostly uniformly colored but sometimes with some indistinct fading zones, bay (a: 19) to umber (a: 18), often sienna (a: 11) towards margin with distinct rust (a: 13) to dark brick (a: 20) or brown vinaceous (a: 25), with broader zonations towards centre and narrower zones towards margin; margin non-striate to very finely and shortly striate, incurved when young, decurved with maturity, becoming very irregularly wavy to undulate. Lamellae broadly adnate to decurrent, crowded (15–16/cm at margin), sometimes forked near the stipe, with lamellulae in 5 series, whitish cream to pale cream, turning livid vinaceous (a: 77) to lilac (a: 79) or darker violet (b: 15D4–5, 15E5) after bruising; edge entire, concolorous. Stipe 35–72 × 10–18 mm, cylindrical or broadest in the middle, narrower towards base, often curved and locally swollen; surface smooth, slightly viscid or sticky, not as viscid as pileus, dingy white to pale cream, buff (a: 52) to vinaceous buff with maturity; base usually paler, strigose. Context firm, rather thick in pileus center and thin in outer half, very soon hollow in stipe, white to pale cream, changing lilac (a: 77; b: 13C3–4) after exposure, leaf green with Guaiac, unchanging with $FeSO_4$ and KOH. Latex white to pale cream, watery, slowly staining the lamellae or context livid vinaceous (a: 77) to lilac (a: 79), unchanging without the contact of lamellae or context. Taste mild at first, becoming slightly bitter and astringent. Odour fruity, very sweet, like pears (as in *Inocybe corydalina* Quél.). Spore print pale cream (c: 10Y).

Basidiospores 8.0–**8.9**–10.5 × 6.9–**7.5**–8.8 µm, (Q = 1.11–**1.18**–1.32), subglobose to broadly ellipsoid, rarely ellipsoid; ornamentation amyloid, ≤1.1 µm high, composed of broad ridges and isolated warts forming a partial to an incomplete reticulum; plage inamyloid or distally amyloid. Basidia 45–52 × 10.5–12 µm, 4-spored, rarely 2-spored, clavate to ventricose; sterigmata 6–7 × 2–2.5 µm. Pleuromacrocystidia 55–80 × 7.5–11 µm, abundant, fusiform with mucronate to moniliform apex, emergent ≤33 µm; content slightly dense. Pleuropseudocystidia cylindric to slightly tortuous, never emergent, 3.5–4 µm wide; content refringent. Lamellar edge sterile. Cheilomacrocystidia 28–43 × 6.5–9 µm, fusiform to lageniform, mostly with moniliform apex. Marginal cells 15–35 × 7–10 µm, cylindric to clavate, slightly thick-walled, hyaline. Subhymenium ≤20 µm thick. Hymenophoral trama with abundant lactifers. Pileipellis an ixocutis to ixotrichoderm, ≤320 µm thick, composed of few repent hyaline hyphae and abundant erect hyphae (2–4 µm broad). Clamp connections absent.

Ecology & Distribution — Gregarious to caespitose under *Abies densa* and *Tsuga dumosa* in subalpine mixed (coniferous and broadleaf) forest. August. Fairly common.

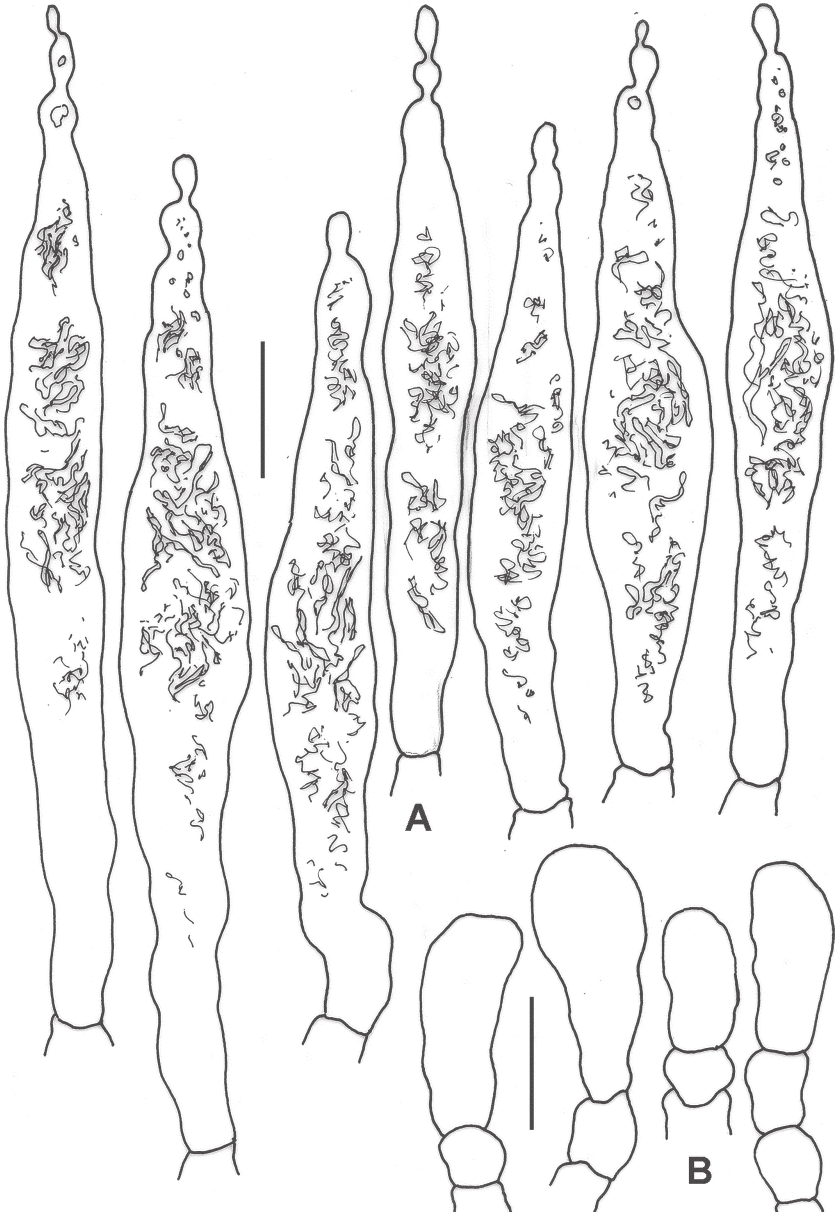

Plate 8. *Lactarius pyriodorus* (Holotype KD 11-027).
A. Pleuromacrocystidia. B. Marginal cells. Scale bars = 10 μm.

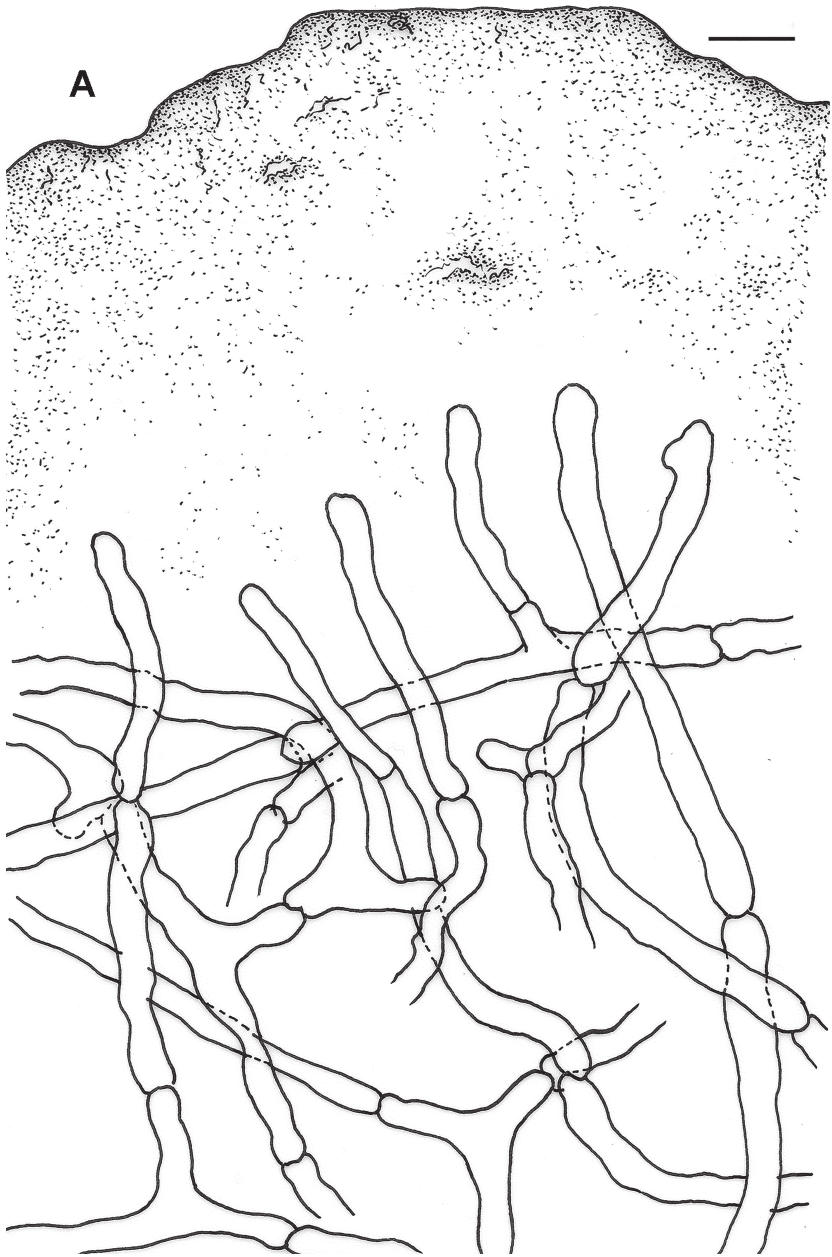

Plate 9. *Lactarius pyriodorus* (Holotype KD 11-027):.
A. Radial section through pileipellis. Scale bar = 10 μm.

Additional specimens examined: **INDIA**. **Sikkim**: North District, Dombang, 27°44′08.7″N 88°45′58.3″E, alt. 2975 m, 14.VIII.2009, A. Verbeken, K. Das & K.V. Putte, AV-KD-KVP 050 & 051 (BSHC, GENT); alt. 2945 m, 23.VIII.2011, K. Das, KD 11-068 (BSHC); North District, Zema, 27°47′20.0″N 88°32′56.1″E, alt. 3079 m, 31.VIII.2012, K. Das, KD 12-223, KD 12-226 (BSHC).

Notes — The viscid to glutinous subzonate pileus, ixocutis to ixotrichoderm nature of the pileipellis, and latex that stains the context and lamellae distinctly lilac to violet place this species in *L.* sect. *Uvidi*. Only one representative of *L.* subg. *Piperites* with lilac milk has been described from Asia (northern Thailand)—*L. formosus* H.T. Le & Verbeken (Le et al. 2007). *Lactarius formosus* clearly differs from *L. pyriodorus* because its pileus is completely covered with bundles of glutinous hairs.

In the ITS-generated phylogenetic tree, the resolution in the group of lilac staining species is very low, and the Indian species is not distinctly separated from a cluster with the European species *Lactarius luridus* (Pers.) Gray and *L. brunneoviolaceus* M.P. Christ. We have previously observed that in this group the morphological variation appears much higher than the ITS molecular variation. Since we observe clear morphological differences between *L. pyriodorus* and the European taxa (also not supported in this tree, although generally accepted as separate species), we choose to present these Indian specimens as a new species. Its darker colours and subzonate aspect are shared with *L. luridus*, which typically associates with broadleaf trees and can be separated by its pileus that is spotted but never with thick gluten (as in *L. pyriodorus*). Also, in *L. luridus,* the stipe base is never strigose and the distinctive sweetish pear-like odour is absent (Heilmann-Clausen et al. 1998). *Lactarius brunneoviolaceus* and *L. pseudouvidus* Kühner are two arctic-alpine species associated with *Salix* that differ greatly from our Indian species in their very small habit. *Lactarius brunneoviolaceus* is further distinguished by its cedar-oil odour and rather large spores (8.8–11.9 × 6.7–8.6 µm) (Heilmann-Clausen et al. 1998, Basso 1999). *Lactarius pseudouvidus* differs microscopically by its distinctly lower (≤0.3 µm) spore ornamentation and differently shaped hymenial cystidia (cylindric to subfusiform with rounded apex) (Heilmann-Clausen et al. 1998). *Lactarius pyriodorus* is superficially similar to the European *L. uvidus* (Fr.) Fr., which can also occur under coniferous trees, but it never has such dark colours (Heilmann-Clausen et al. 1998), and its spores have rounded warts and ridges forming a more incomplete reticulum.

This is for the first time that distinct sweetish odour reminiscent of pears (also known from the European *Inocybe corydalina*) has been encountered in *Lactarius*.

Lactarius yumthangensis K. Das & Verbeken, **sp. nov.** PLATE 10–12, 13C,D,F

MYCOBANK MB 804890

Differs from *Lactarius trivialis* by its lighter spore print, smaller spores, and pileipellis ixocutis.

TYPE: India. Sikkim: North District, Yumthang valley of Shingba Rhododendron sanctuary, 27°46′51.9″N 88°42′37.6″E, alt. 3586 m, 30.VIII.2011, K. Das, KD 11-147 (**Holotype**, BSHC, **isotype**, GENT).

ETYMOLOGY: after the name of the locality from where the type specimen was collected

PILEUS 37–98 mm diam., at first convex with inrolled margin, becoming planoconvex to applanate with depressed indented centre or funnel shaped at maturity, surface smooth, viscid when dry, slimy when moist, vinaceous grey (a: 80) or slightly paler (purplish grey), mostly with distinct darker spots forming zonations; margin non-striate, paler, decurved with maturity. LAMELLAE broadly adnate to decurrent, close to medium crowded (9–11/cm at margin), some forked, with lamellulae in 5 series, cream-yellow to yellow (a: 6F) or ochraceous, sometimes with rusty spots after maturity, unchanging when bruised; edge entire, concolorous. STIPE 21–50 × 9–22 mm, cylindrical to ventricose, often constricted below the juncture of the lamellae, surface slightly longitudinally venose, whitish on constriction, then vinaceous to vinaceous grey (a: 80), cream yellow towards base, with ochraceous areas/spots towards base after maturity. CONTEXT yellowish white to cream, hollow, multi-chambered in stipe, becoming greenish with $FeSO_4$ and orange-yellow with KOH. LATEX chalky white, changing to orange-yellow with KOH, turning pale cream (a: 3C) after some time, slowly (on drying) greenish yellow (a: 57) after 2 hours (on cut lamellae). TASTE acrid. ODOUR fruity. SPORE PRINT pale yellow (c: 30Y, 2M).

BASIDIOSPORES 6.4–**7.1**–8.0 × 5.3–**6.0**–6.9 µm, (Q = 1.06–**1.18**–1.32), subglobose to ellipsoid; ornamentation amyloid, ≤1 µm high, composed of rather regular, narrow and acute ridges mostly arranged in parallel groups forming zebroid pattern, short ridges and isolated irregular warts present between ridges, but, never forming reticulum; plage amyloid distally but often not distinguishable. BASIDIA 38–58 × 9–11 µm, 2–4-spored, clavate to ventricose; sterigmata long, 5–9 × 2–2.5 µm. PLEUROMACROCYSTIDIA 44–90 × 7.5–10 µm, abundant, clavate to fusiform (sometimes with mucronate apex), emergent ≤36 µm; content slightly dense but never needle-like, slightly thick-walled (wall up to 0.6 µm). PLEUROPSEUDOCYSTIDIA irregularly tortuous with rounded apex, 2.5–4 µm wide; contents dense. LAMELLAR EDGE sterile. CHEILOMACROCYSTIDIA 27–55 × 8–9 µm, subclavate or narrowly clavate to fusoid, emergent ≤25 µm, slightly thick walled (wall ≤0.7 µm). MARGINAL CELLS forming chains of elements, terminal cells 11–18 × 5–8 µm, subcylindric to subclavate or clavate, hyaline. SUBHYMENIUM ≤28 µm thick, cellular.

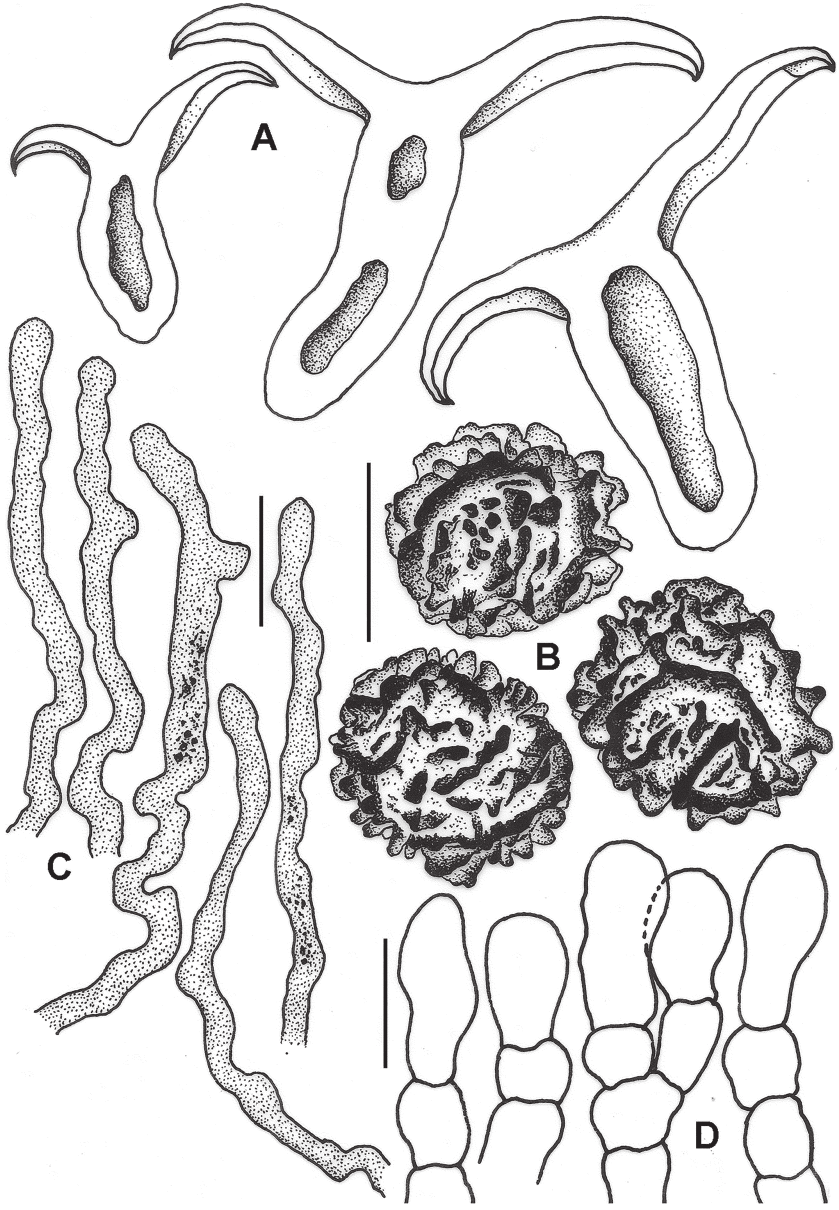

Plate 10:. *Lactarius yumthangensis* (Holotype KD 11-147).
A. Fresh basidiomata showing lamellae and lamellulae. B. Basidiospores.
C. Pleuropseudocystidia. D. Marginal cells. Scale bars: B = 5 µm; C, D = 10 µm.

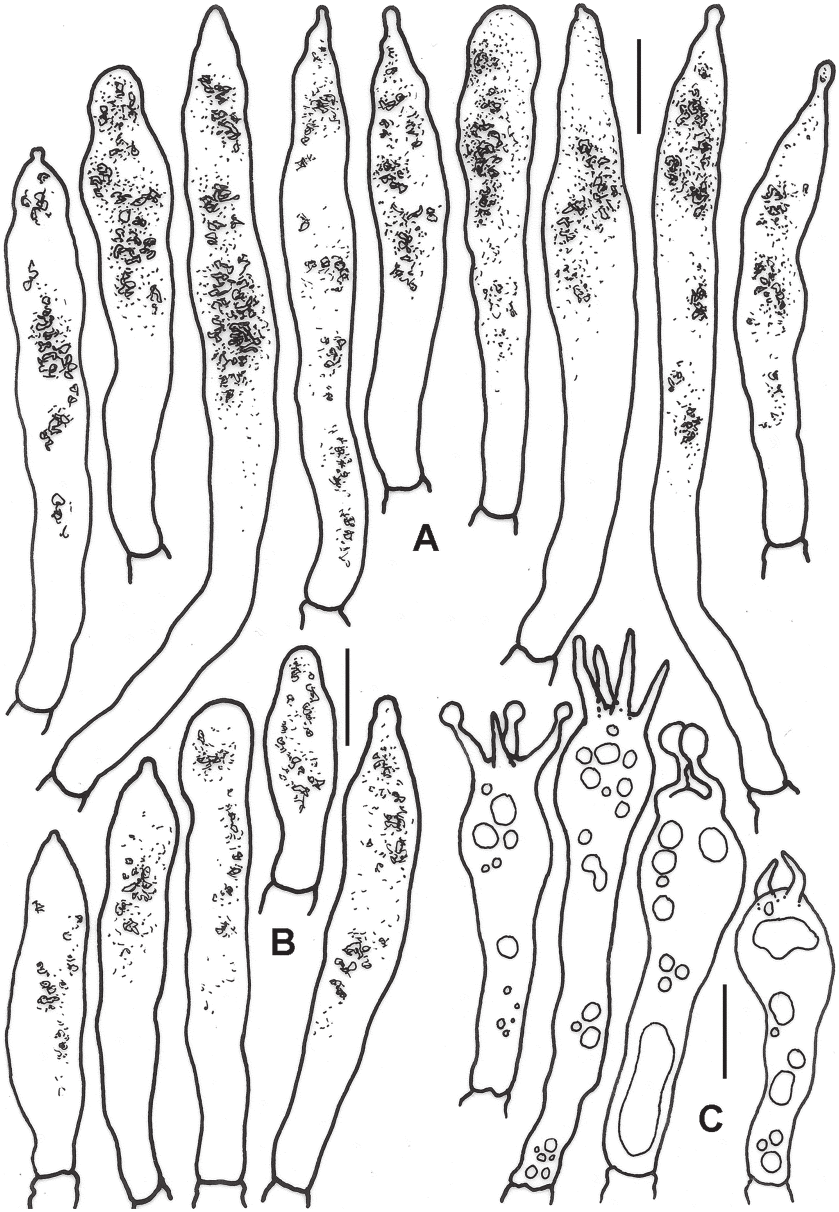

Plate 11. *Lactarius yumthangensis* (Holotype KD 11-147).
A. Pleuromacrocystidia. B. Cheilomacrocystidia. C. Basidia. Scale bars = 10 µm.

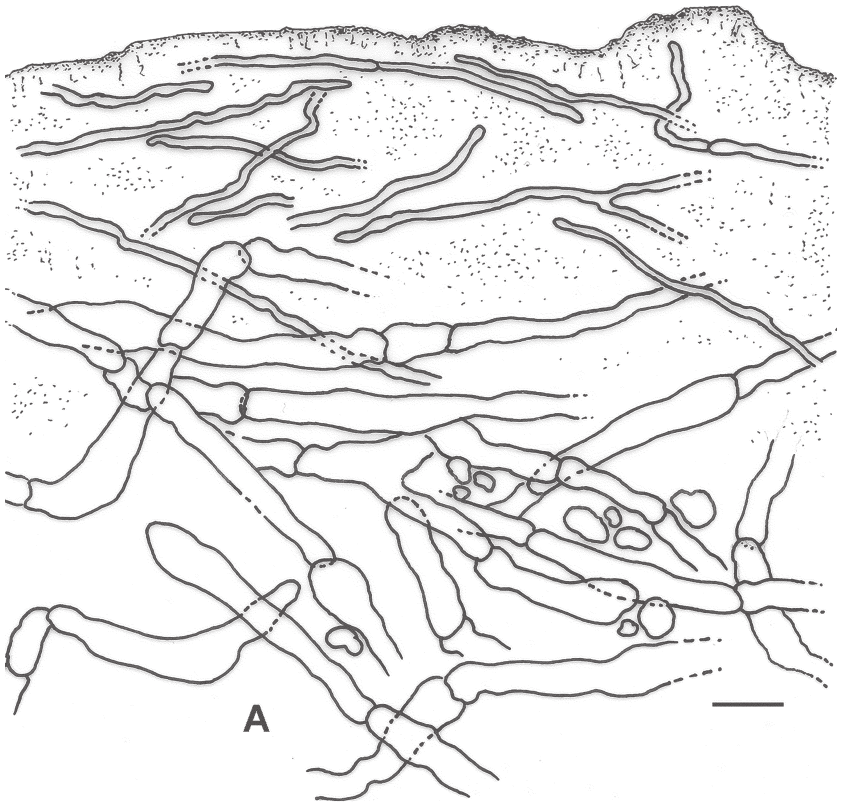

Plate 12. *Lactarius yumthangensis* (Holotype KD 11-147).
A. Radial section through pileipellis. Scale bar = 10 μm.

Pileipellis an ixocutis, composed of narrow repent hyaline hyphae and broad (≤7 μm) septate hyphae embedded in a layer of slime. Clamp connections absent.

Ecology & Distribution — Gregarious to caespitose under *Betula utilis* in subalpine mixed (coniferous and broadleaf) forest. August–September. Uncommon.

Additional specimens examined: **India**. **Sikkim**: North District, Yumthang valley of Shingba Rhododendron sanctuary, 27°46′51.9″N 88°42′37.6″E, alt. 3586 m, 29.VIII.2011, K. Das, KD 11-150 (BSHC); 27°45′11.1″N 88°43′47.5″E, alt. 3580 m, 01.IX.2011, K. Das, KD 11-172 (BSHC).

Notes —The viscid to slimy zonate pileus and the ixocutis to ixotrichoderm nature of the pileipellis undoubtedly place the present species in *L.* subg.

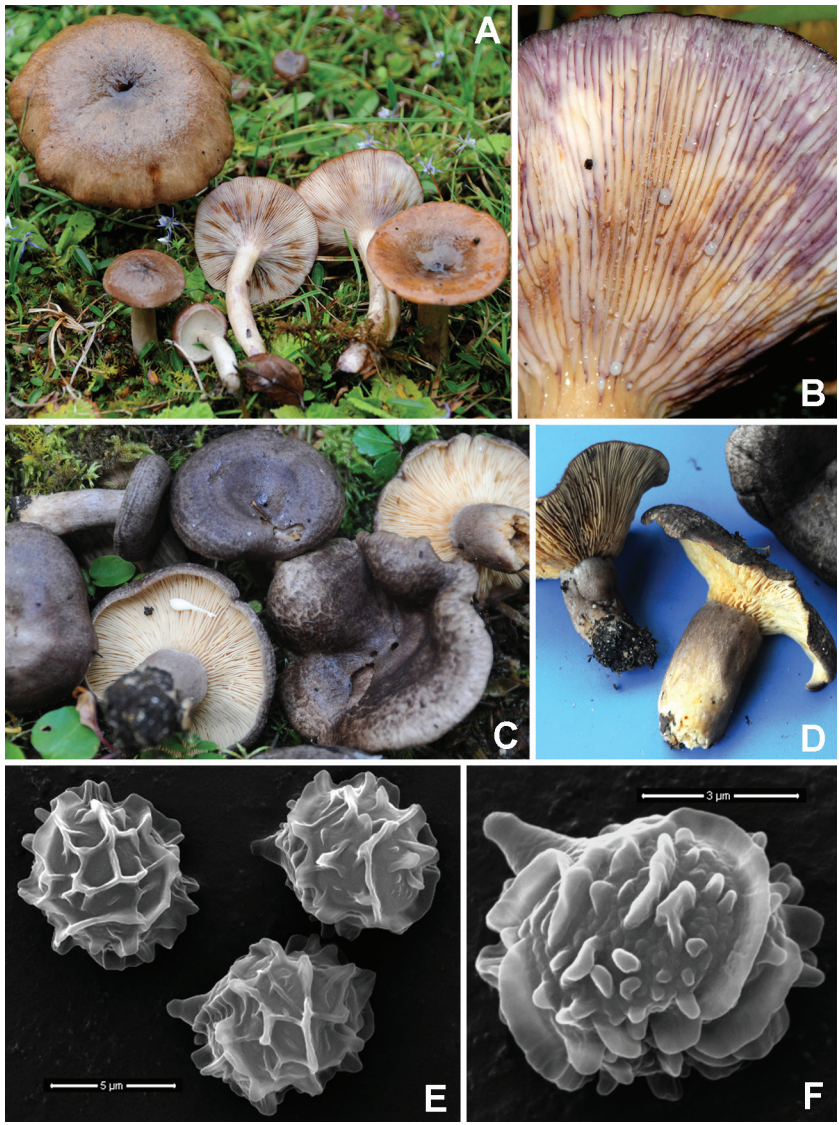

Plate 13. ***Lactarius pyriodorus***. A. Fresh basidiomata. B. Latex oozes out from cut lamellae. E. Basidiospores (SEM). ***Lactarius yumthangensis***. C. Fresh basidiomata. D. Basidiomata showing constricted stipe-apex. F. Basidiospores (SEM). Scale bars: E = 5 µm; F = 3 µm.

Piperites. *Lactarius yumthangensis* can be recognized by its vinaceous grey to purplish grey pileus, venose stipe with a constricted apex, chalky white latex

that changes to orange-yellow with KOH and becomes greenish yellow after long exposure, and growth under *Betula* sp.

Morphologically, *L. flexuosus* (Pers.) Gray and *L. trivialis* (Fr.) Fr. (both reported from Europe) appear quite similar. However, both can be distinguished from *L. yumthangensis* by the slightly darker spore print (c: 40Y, 5M). Moreover, the white latex of *L. flexuosus* is unchanging (never becoming greenish yellow after exposure), the stipe is not constricted at the apex (Kränzlin 2005), and some cheilomacrocystidia have a moniliform apex (Heilmann-Clausen et al. 1998). In *L. trivialis*, spores are distinctly larger (7.3–10 × 5.9–7.8 µm) and the pileipellis is an ixocutis to ixotrichoderm (Heilmann-Clausen et al. 1998).

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